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ST486 cells, but not in the drug resistant EW36 and CA46 cell lines. However, mClCCP was 2-fold more potent (60% *versus* 30%) than ATA for inducing apoptosis. Each inhibitor in ST486 cells differentially induced the MAP kinases ERK and p38. Furthermore, mClCCP induce 3-fold more phosphorylated ERK than induced by ATA. This differential potency was also evidenced by the 2.3-fold more p38 activity induced by mClCCP and the 3-fold more phosphorylation of the p38 substrate ATF2. These results support a role for mitochondrial regulation of drug sensitivity involving activation of ERK and p38 and differential potency of mitochondrial inhibitors related to the degree of activation of these MAP kinases.

1340 MECHANISMS OF IMMUNOSUPPRESSION BY ORGANOTINS – APOPTOSIS *vs.* PROLIFERATIVE ARREST.

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The organotin compounds di-n-butyltin dichloride (DBTC) and tri-n-butyltin chloride (TBTC), used as stabilizers and biocides respectively, induce thymus atrophy inhibiting immature thymocyte proliferation. The aims of the study were to examine whether apoptosis has a role in this atrophy and whether DBTC, like TBTC, induces apoptosis *in vitro*. Thymi from rats treated with a dose (15 mg/kg) of organotin known to reduce thymocyte proliferation, did not show DNA fragmentation, indicating that apoptosis is not evident in organotin-induced thymus atrophy at low doses. *in vitro*, data showed that 3-5 uM of DBTC or TBTC significantly increased the percentage of apoptotic nuclei in rat thymocytes. Further mechanistic studies indicated a relation between the cytotoxic effects of the compounds and their capacity to induce apoptosis. At lower concentrations than required to induce apoptosis, both organotins inhibited protein and DNA synthesis and increased RNA and heat shock proteins synthesis. We demonstrated that the increase of RNA synthesis occurred in small thymocytes, which comprised the same subset of cells sensitive to apoptosis by organotins. Moreover, co-exposure to RNA or protein synthesis inhibitors protected cells from apoptosis by DBTC or TBTC, indicating that macromolecular synthesis is required for the initiation of the process. Besides effects on macromolecular synthesis, organotins disrupt energy metabolism and affect mitochondria. Previously, TBTC has been shown to increase intracellular calcium level, to produce reactive oxygen species (ROS) and to release pro-apoptotic factors. We showed similar changes in case of DBTC, *i.e.*, increase of calcium, ROS production, release of cytochrome c and activation of caspase 3. Thus, induction of apoptosis is a relevant mechanism at relatively high concentrations/doses of organotin compounds, while lower concentrations/doses cause a proliferative arrest without signs of apoptosis.

1341 EXPOSURE TO PESTICIDES INDUCES APOPTOSIS IN SPLENOCYTES.

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Alachlor and fenthion are extensively used as pesticide mixtures throughout the world and present a potential health risk to human and the environment. The immunotoxic potential of these pesticides and pesticide mixtures is currently unknown. We examined the effects of pesticide mixtures in inducing apoptosis in splenic immune cells of C57BL/6 male mice *in vitro*. Splenocytes were subjected to minimal pesticide concentrations and exposure time necessary to cause detectable cell damage to parameters often associated with apoptosis. The results of TUNEL assays and DNA ladder assays indicated an approximate 20% increase in degradation of high molecular weight genomic DNA after 4 hours exposure in cells treated with either 100μM fenthion, 100 μ M alachlor or 100μM each of these pesticides in mixture. The annexin assays show a 40 –50% increase in cell membrane damage in cells treated with either 100 μ M alachlor, 100μM fenthion or 100 μ M each of pesticide mixture for 4 hours *versus* that seen in untreated cells ($p \leq 0.01$). LDH assays also show an increase of 15% ($p \leq 0.01$) cell membrane damage over untreated cells that was first detected after a 4 hours exposure to either 100 μ M alachlor or 100 μ M fenthion. These data indicate that immune cells exposed to alachlor, fenthion, and alachlor /fenthion mixtures sustain cell damage that can be associated with apoptosis. Both DNA and membrane damage continues to increase in a dose (10-250 μ M) and time (0-16 hr) depended manner. There was no significant increase in either DNA damage or cell membrane damage when cells were exposed to mixed pesticides *versus* that observed when cells were exposed to individual pesticides.

1342 EFFECT OF DIETARY N-ACETYL-L-CYSTEINE (NAC) ON APOPTOSIS AND MITOGENESIS IN SPLENIC LYMPHOCYTES AFTER ACUTE RADIATION EXPOSURE.

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Apoptosis and cellular proliferation are pivotal opposing processes that regulate cell number in lymphoid tissues. Supplementation with antioxidants, such as NAC, may affect these processes and alter responses to stressors. To test this hypothesis, we irradiated mice and assessed splenic responses to mitogen stimulation. Female FVB/N mice were fed purified basal diet alone (CTL; n=16) or containing 3% NAC (NAC; n=16) for 2 wk prior to irradiation with an acute dose of 4 Gy. Subsequently, mice (n=4/diet/time point) were killed by CO2 narcosis at 3, 6, 9, and 18 h post-irradiation. Spleen to body weight ratios declined linearly over 18 h from 3.7 to 2.3 mg/g as did cellularity from 0.6 to 0.1 (x10e6) cells/mg spleen with no differences between dietary groups. Splenocytes were isolated and cultured in medium alone or containing increasing concentrations of T cell-specific Con A (0.3-30 ug/ml) or B cell-specific LPS (3-300 ug/ml) for 72 h. Following Con A stimulation, mitogenesis increased 300% in splenocytes from CTL mice, but only 50% in cells from mice on NAC supplementation. Mitogenesis in CTL splenocytes was suppressed for at least 6 hours when incubated with LPS, but recovered fully by 9 h. Similarly treated splenocytes from NAC supplemented mice did not recover until 18 h later. Apoptosis (TUNEL) was increased in Con A-stimulated CTL and NAC cells at 3 h but returned to baseline by 6 h. In LPS-exposed CTL cells, apoptosis increased maximally at 6 h. In cells from NAC-fed mice, the increase in LPS-induced apoptosis occurred after 3 h, peaked at 9 h, and remained elevated up to 18 h. Analysis by flow cytometry using B and T cell specific CD45R-FITC and CD3-PE monoclonal antibodies, indicated marked depletion of B cells in both CTL and NAC cultures but survival of T cells over 18 h with no difference between groups. The results suggest that NAC can alter the temporal response profile of murine splenocytes to mitogens after an acute genotoxic stressor.

1343 EFFECTS OF METHOMYL ON CELL CYCLE AND APOPTOSIS IN HUMAN HEMATOPOIETIC CELL LINES.

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Methomyl is an anti-acetylcholinesterase insecticide which reduces cell viability, induces oxidative stress, and structurally alters tubulin and the phosphodiester group in nucleic acids in rat spleen cells. These effects show that methomyl probably causes toxicity in hematopoietic cells which are the major spleen cell type. The ability of methomyl to induce apoptosis on human hematopoietic cell lines *in vitro* was investigated and quantified using three parameters:1) flow cytometric detection of sub G1 population in propidium iodide stained cells, 2) detection of exposed phosphatidyl serine on the plasma membrane using annexinV-FITC, and 3) detection of changes in mitochondrial transmembrane potential using tetramethylrhodamine ethyl ester. The results showed that methomyl caused apoptosis in MM6, a human mature monocytic cell line; THP1, a human monoblastic cell line; and Jurkat, a human leukemic T cell line. The sensitivity of each cell type to methomyl varied. Methomyl induced apoptosis in MM6, THP1, and Jurkat cells at final concentrations of 6, 1.5, and 6 mM, respectively, after a 24 hour exposure. In addition, the cell cycle profiles after methomyl exposure were also different in each cell type. MM6 and Jurkat cells showed a significant increase in the S phase before undergoing apoptosis while THP1 cells did not. However, methomyl did not induce apoptosis in Raji, a human lymphoma B cell line, but did cause a cell cycle arrest in the G0/G1 phase. The different sensitivities and cell cycle profiles of each cell type in response to methomyl treatment are possibly dependent upon differential cell surface antigenic expression. The mechanisms as to how methomyl causes apoptosis in hematopoietic cells are still unknown but alterations in cellular signal transduction which could trigger the apoptotic process in these cell types following methomyl exposure are currently being investigated.

1344 APOPTOSIS IN TESTES INDUCED BY CO-EXPOSURE OF RATS TO DEET, PERMETHRIN AND PYRIDOSTIGMINE BROMIDE ALONE, AND IN COMBINATION WITH STRESS.

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Male Sprague-Dawley rats were treated with a combination of DEET (40mg/kg, dermal), permethrin (0.13mg/kg, dermal), and PB (1.3mg/kg, oral) with and without stress for 28 days. The animals were subjected to stress by putting them in a

Plexiglas® restrainer (5 minutes/day). 24 Hrs after the last treatment, one set of animals was given single i.v. injection of [^{3}H]hexamethonium iodide to evaluate blood-testis barrier (BTB). A second set of animals was perfused with 4% paraformadehyde and the testes were dissected out for histopathological and immunohistochemical evaluations. Significant increase in testicular [^{3}H]hexamethonium iodide uptake was observed in the animals treated with the combination of chemicals and this uptake was further enhanced in the animals treated with combination of chemicals and stress. Severe damage to somniferous tubules by H&E staining was observed in the animals treated with both the combination of chemicals, and in combination with stress. Extensive immunostaining with monoclonal antibodies against single stranded DNA was observed in the testes from the animals treated with combination of chemicals that was further increased with the combination of chemicals and stress exposure. Furthermore, an increase in Bax immunostaining was observed in animals treated with either chemicals alone or in combination with stress. These results suggest that apoptosis may play a major role in testicular degeneration following combined exposure to real-life levels of PB, DEET, and permethrin with stress. Supported, in part by the U.S. Army Medical Research and Materiel Command under contract # DAMD 17-99-1-9020. The views, opinion and/or findings contained in this report are those of the authors and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

1345 EARLY CHANGES IN FAS/FAS L, DR4&DR5/TRAIL, AND CASPASE-8 ACTIVITY INDICATE BOTH FAS- AND FAS-INDEPENDENT SIGNALING IN TESTES OF C57BL/6 AND GLD MICE EXPOSED TO MONO-(2-ETHYLHEXYL) PHTHALATE.

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Exposure of C57BL/6 (C57) mice to the Sertoli cell toxicant mono-(2-ethylhexyl) phthalate (MEHP) results in an increased incidence of germ cell (GC) apoptosis which is not observed in Fas ligand (FasL) defective (gld) mice. To confirm the role of Fas signaling in GC apoptosis and to determine whether other pathways independently cause GC death we: (1) measured caspase-8 activity in whole testis homogenates after exposing 28 day-old mice to MEHP; and (2) assessed protein changes in FasL (membrane and soluble [sFasL]), Fas receptor (Fas), death receptors 4 and 5 (DR4/DR5), and TRAIL. Following MEHP exposure, C57 mice showed an initial 5-fold increase in Fas by 3 h, a brief decline and then a gradual increase (two-fold by 12 h). After an initial increase, Fas levels declined in gld testes. However, DR4 and DR5 protein levels increased in testes of both strains by 12 h, but more so in the gld mice. Contrary to Fas induction, but similar to previously reported apoptosis trends observed in rat and mouse GCs, levels of active caspase-8 initially dropped below control in both strains before rebounding above control by 6 h (C57) and 3 h (gld). FasL/sFasL levels increased in C57 testes by 1.5 h and then returned to basal levels. Levels declined at the earliest time point (1.5 h) in gld testes. Conversely, levels of TRAIL increased sooner (1.5 h) and were comparatively higher in gld *vs.* C57 mice. These data show that Fas induction occurs earlier and to a greater degree than previously reported. Moreover, the delay between early Fas induction *versus* caspase-8 activation and DNA fragmentation in C57 mouse testes supports the hypothesis that Fas is the primary initiator of testicular germ cell apoptosis after MEHP exposure. Furthermore, observations with the gld strain indicate a role for Fas-independent apoptotic signaling in mouse testes. (Supported by R01ES09145 and Center Grant ES07784).

1346 INCREASED SENSITIVITY TO TNF-α-INDUCED APOPTOSIS IN CELLS LACKING MITOCHONDRIAL DNA.

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Mitochondria play pivotal roles in apoptosis. Our previous results showed that cells lacking mitochondrial DNA (ρ^0 cells) retain complete apoptotic machinery and have similar response to staurosporine-induced apoptosis as compared to ρ^+ cells. In this study, we looked how ρ^0 cells respond to tumor necrosis factor-α-induced apoptosis. 143B osteosarcoma ρ^0 and ρ^+ cells were treated with 20 ng/ml TNF-α, and apoptosis was measured by Annexin V staining followed by flow cytometry. Caspase activity was measured with fluorogenic substrates. Results showed that ρ^0 cells had a higher percentage of apoptotic cells after 12 hr treatment and they had a much more rapid and potent activation of effector caspases. Intracellular GSH/GSSG redox potential in ρ^0 cells was largely unchanged with an 8 hr treatment, while ρ^+ cells were considerably oxidized. We conclude that ρ^0 cells are more sensitive to TNF-α-induced cytotoxicity which could involve a mitochondrial electron transport chain-dependent protective mechanism against apoptosis. (Supported by NIH grant ES09047).

1347 TRANSLATION REPRESSOR 4E-BP1 ACTIVATED APOPTOSIS DEPENDS ON ITS PHOSPHORYLATION STATUS.

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Translation rates generally increase by growth factors, cytokines, hormones and mitogens. Regulation of translation mainly occurs at the level of initiation. Eukaryotic translation initiation factor 4E (eIF4E) is the mRNA cap binding protein which functions during translation of cellular mRNAs possessing the 5' cap structure. Overexpressed eIF4E suppresses oncogene-dependent apoptosis, causes malignant transformation and leads to multi-drug resistance. The function of eIF4E is negatively regulated in part by members of the family of translational repressors, eIF4E-binding proteins 4E-BPs. When hypophosphorylated, 4E-BPs block cap-dependent translation by sequestering eIF4E in a translationally inactive complex. Upon hyperphosphorylation in response to hormones or growth factors, 4E-BPs dissociate from the complex with eIF4E allowing it to form an active translation initiation complex. Previously, we found that overexpression of eIF4E blocks Myc-induced apoptosis whereas enforced expression of 4E-BP1 promotes both spontaneous and drug-induced apoptosis in Ras-transformed fibroblasts *in vitro* and diminishes tumorigenicity of oncogenic Ras in vivo. Here we show that ectopic expression of 4E-BP1 activates apoptosis. Rapamycin, an inhibitor of the signaling pathway leading to phosphorylation of 4E-BP1, augments its pro-apoptotic function. Furthermore, we demonstrate that mutations of 4E-BP1 decreasing or eliminating its phosphorylation significantly potentiate spontaneous and drug induced apoptosis in normal and Ras-transformed fibroblasts and dramatically reduce their colony forming efficiency. These data suggest that phosphorylation of 4E-BP1 through a rapamycin-sensitive kinase cascade promotes cell viability and increases resistance to anti-cancer therapy.

1348 CYTOCHROME C RELEASE AND SUBSEQUENT ACTIVATION OF CASPASE-3 IS INVOLVED IN CYANIDE-INDUCED APOPTOSIS.

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Both *in vitro* and *in vivo* studies suggest that cyanide induces apoptosis in cortical neurons. To identify the mechanistic pathways leading to apoptosis, primary cultured cortical cells were used to study cyanide cytotoxicity. Caspase activation is a characteristic criterion of apoptosis. After cyanide treatment (100-300 μM) for 24 hrs, caspase-3 was cleaved to its active form as detected by western blot analysis. Poly(ADP-ribose) polymerase (PARP), an important substrate of caspase-3, was activated after cyanide treatment, further confirming that caspases were activated during cyanide-induced apoptosis. Cytochrome c release from mitochondria is one pathway that has been identified to activate the caspase cascade. After 300 μM cyanide treatment for 3 hr, cytochrome c was released into cytosol and it remained there for up to 24 hrs as detected by western blot analysis. NMDA receptor activation and reactive oxidative species (ROS) generation were upstream events of cytochrome c release, since the selective NMDA receptor antagonist MK801 and the antioxidant PBN (N-tert-butyl-phenylnitrone) partly blocked cytochrome c release from the mitochondria. Also mitochondria depolarization plays an important role in cytochrome c release, since immediately after cyanide treatment, the mitochondria membrane was depolarized by cytofluorometric analysis of cells stained with rhodamine 123 and blockade of mitochondria depolarization by cyclosporin A partly blocked cytochrome c release. Z-VAD, a caspase inhibitor, had little effect on cytochrome c release, further confirming that caspase activation was downstream of cytochrome c release. These results show that cytochrome c release from mitochondria plays an important role in cyanide-induced apoptosis, and mitochondria depolarization contributes in part to the release of cytochrome c. (Supported by NIH grant ES04140)

1349 ACTIVATION OF OXIDATIVE STRESS-DEPENDENT CELL SIGNALING PATHWAYS IN METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL (MMT)-INDUCED APOPTOSIS: DOWNSTREAM EVENTS AND REGULATORY MECHANISMS.

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It is well known that chronic manganese intoxication can lead to Manganism, a neurological condition similar to Parkinson's disease. Despite the known neurotoxic effect of manganese on the dopaminergic system, MMT has recently been legalized